TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

#86AR1
(Originally activated Oct. 1, 1956)

Application For Research Grant

Date:

July 15, 1957

1. Name of Investigator:

Sydney C. Rittenberg, Ph.D.

2. Title:

Professor of Bacteriology

3. Institution & Address:

University of Southern California, Los Angeles 7, California.

4. Project or Subject:

The bacterial degradation of nicotine and related compounds. Studies on the fate of the products of nicotine degradation.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

The plan of procedure to be employed in the proposed research program will be essentially the same as the one applied to the investigations now in progress. The successful application of the classical methods of enzymology to the elucidation of the primary step of nicotine oxidation makes it likely that a similar approach will be successful in working out the subsequent steps.

As was mentioned in the first annual report, a crude cell-free system has been obtained that carries nicotine oxidation to the 3 μm level (i.e., to a product accumulating after 3 μm of oxygen have been consumed per μm of nicotine). The crude extract has been partially fractionated to give preparations that cause the accumulation of the first and second oxidation products and the first product has been isolated and tentatively identified. We have indications that the second product can be isolated in a manner similar to the first, that is by precipitation with silicotungstic acid followed by ion-exchange chromatography using increasing concentrations of potassium chloride as the eluting agent. The next goal we have set is the isolation and identification of the second product and this may be achieved before the termination of the current grant.

The ~~gross~~ properties of the available enzyme fractions are such that it is certain we can cause the accumulation of the 3 μm oxidation product and almost certain that the same can be done for the 1.5 μm product without further fractionation procedures. Following the identification of the second step product we propose to go after these two products next. It is not certain at this time whether the same methods of isolation now employed for product one and two will be suitable for subsequent products since obviously the chemistry of each succeeding product will differ; consequently the methods to be employed will have to be chosen or developed as we go along.

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5. Detailed Plan of Procedure. (Cont'd)

For the fractionation of the crude enzyme system, we have only employed a single method so far (ammonium sulfate fractionation) and even this procedure has not been studied exhaustively. Consequently, after the products now available have been obtained further fractionations of the crude extract will be attempted using not only ammonium sulfate precipitation but also other procedures already available for this purpose. With complete success the first six oxidation products could be obtained; it is obviously impossible however to predict how successful we shall be.

In addition to the products now available by use of the enzyme preparation, the 7th product (s) are also available by means of dry cell oxidation. Since this product (s) results from an extensive breakdown of the nicotine molecule with a release of about 50 percent of the initial carbon as CO_2, it (they) should be a relatively small molecule and its identification by paper chromatography will be attempted.

It should also be mentioned that enzymes capable of carrying nicotine oxidation to levels between the 3 and 7th stage might be obtainable from the dry cells or from fresh cells using different methods of extraction. Depending on progress along the avenues now open, attempts may be made to obtain the intermediate enzymes and thus make possible the isolation and identification of additional molecules in the oxidation sequence.

The final logical step would be the reconstruction of a complete cell-free system capable of oxidizing nicotine or any of the intermediates to the terminal stage of nicotine degradation. We are not so sagacious as to imagine this could be achieved within the next year but in view of the success that the enzymological approach has had in the current program continued progress is anticipated.

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Source: https://www.industrydocuments.ucsf.edu/docs/kppl0000

6. Budget Plan:

Salaries	
Expendable Supplies	5,000
Permanent Equipment	1,500
Overhead	- -
Other (8%) (Travel)	544 300
Total	7,344

7. Anticipated Duration of Work:

1 year

8. Facilities and Staff Available:

All the equipment required for the investigations is available in our laboratory. No "staff" beyond the project director and a post doctoral fellow is required. The salary item in the budget is for a post doctoral fellow at full time.

9. Additional Requirements:

None.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The relation of this problem to investigations of other contemporary workers is difficult to assess at the present time. To date, no investigations dealing with the intermediary metabolism of nicotine at the enzymatic level have been reported in the literature. This seems strange and such investigations would be in order in view of the increased proportion of the population coming into contact with nicotine. Certainly its metabolic fate poses a problem possessing more than an academic interest.

On the other hand, a number of papers have appeared dealing with what may be termed the "gross metabolism" of nicotine. These papers may be divided into two classes; mixed culture studies, and growth studies employing pure cultures. The methodology common to both these approaches has been the isolation of substances arising during growth at the expense of nicotine. From the chemical nature of these compounds, pathways were constructed based upon a logical sequence as determined by the structures of the substances. These results, valuable as they are, do not in themselves give any clue as to whether these compounds represent major pathways of degradation or are (see following page)

Signature _____
Director of Project /s/ Sidney C. Rittenberg

Business Officer of Institution /s/ Robert B. Fisher

Financial Vice-President

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10. Additional Informatio
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"metabolic dead ends." Nor da
may determine which metabolic s

The value of a research pi
in this laboratory, has been enum
request; no reason to change these
at the present time.

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